

# Selective enrichment of ESBL, AmpC and carbapenemase producing E. coli in meat and cecal samples - additional validation for poultry samples

**Cavaco, Lina; Hendriksen, Rene S.; Agersø, Yvonne; Svendsen, Christina Aaby; Nielsen, H.; Guerra, B; Peran, R.; Hasman, H.**

*Publication date:*
2016

*Document Version*
Publisher's PDF, also known as Version of record

**Background:** In the European Union a mandatory specific monitoring of extended-spectrum (ESBL) and AmpC beta-lactamase producing *Escherichia coli* (Commission Implementing Decision 2013/652/EU) has started in 2015 with porcine and bovine. A protocol was designed and validated for this purpose on samples from bovine and porcine origin. Because starting in 2016 this protocol should be applied to poultry samples, we performed a validation of the pre-existing method using matrices of poultry origin (chicken meat and chicken caecal content).

**Material/methods:** *E. coli* isolates producing the following ESBL/AmpC/carbapenemase were included in the experiments: CTX-M-1, CMY-2, VIM-1 and OXA-48 and the ATCC 25922 control strain were used. Bacterial suspensions were added (spiked) to the matrices in concentrations of 0.1 (only meat samples), 1, 10, 100 and 1000 CFU/gram matrix sample. All methods included a pre-enrichment step in Buffered Peptone Water (BPW) and incubation over night at 37°C. Subsequently semi-quantitative plating on MacConkey agar supplemented with 1 mg/L of cefotaxime was performed and incubated at 44°C. Additionally the pre-enrichment was plated similarly on Chrom ID CARBA and Chrom ID OXA-48 specific plates for detection of carbapenemase carrying strains. The caecal samples were additionally plated on TBX agar and EMB agar plates both supplemented with 1 mg/L cefotaxime for comparison.

**Results:** The pre-enrichment using BPW allowed detecting *E.coli* producing CTX-M, CMY-2 and VIM-1 on the plates supplemented with cefotaxime. The detection limit found was about 0.1 CFU/g in chicken meat samples and 10-100 CFU/g in chicken caecal samples. Incubation of the MacConkey plates at 44°C reduced the background flora, facilitating the detection.
For the caecal samples the detection limits were similar for MacConkey, TBX or EMB agar, however, it was observed that some of the strains did not produce blue colonies on TBX agar as expected for *E. coli* and that in, general, the background on the EMB agar plates was more abundant.
The *E. coli* strain producing OXA-48 and the susceptible control strain were not detected on the plates supplemented with cefotaxime, as expected; however the OXA-48 positive strain could be detected by performing additional plating on ChromID OXA-48 plates. The samples carrying VIM-1 producing strains could be detected on the MacConkey agar plates with cefotaxime and on the ChromID CARBA plates.

**Conclusions:** The protocols to specifically select for *E. coli* ESBL/AmpC producers in meat and cecal samples were validated to be used for samples of poultry origin including a pre-enrichment step without antibiotics in BPW and subsequent plating on selective MacConkey agar plates containing 1 mg/L cefotaxime, followed by incubation at 44°C. This method was able to detect *E. coli*-producers of ESBL or AmpC producers. For OXA-48 and carbapenemase detection the method can be extended by adding specific carbapenemase and OXA-48 detection agar plates.